EXPERIMENTS ON THE TREATMENT OF ANIMALS INFECTED WITH TRYPANOSOMES BY MEANS OF ATOXYL, VACCINES, COLD, X-RAYS AND LEUCOCYTIC EXTRACT; ENUMERATIVE METHODS EMPLOYED*

BY

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These experiments were conducted with funds given by Sir Edwin Durning-Lawrence, Bart., for the purpose of testing the effect of cold on disease, and were suggested by one of us (R.R.) as a part of the studies made in connection with his case of sleeping sickness, as reported upon in an accompanying paper by him and Dr. David Thomson. Although many researches have been made on the effect of atoxyl and other drugs, we believe that these are the first in which that effect has been measured by regular daily counts of the parasites by measured thick-film methods.

I. ATOXYL

(a) Small doses.

From a study of the patient, W.A., at the Royal Southern Hospital by Major R. Ross and D. Thomson it will be noted that atoxyl failed to be of any marked benefit to the patient, and in the doses administered there was no trypanocidal action detected. It was necessary, therefore, to begin a series of experiments on animals inoculated with *T. rhodesiense* (Stephens and Fantham †),

* An abstract of this paper was read before the Royal Society on December 8th, 1910, and published in Proc. Roy. Soc., B, Vol. LXXXIII, pp. 227-234 (1911).

† Proc. Roy. Soc., Series B, Vol. LXXXIII, pp. 28-33 (1910), and Ann. Trop. Med. and Parasit., Vol. IV, pp. 343-351.

and to try the effect of small doses of atoxyl in these animals with a view to determine the effect of that drug on this particular trypanosome which, as will be seen from the accompanying paper on 'Enumerative Methods in Untreated Animals' (H. B. Fantham and J. G. Thomson*), was of extraordinary virulence. Various doses of atoxyl are recommended in treatment of human trypanosomiasis, and we find that the patient, W.A., in the Royal Southern Hospital, received four grains as a maximum dose. It was impossible in the case of this patient to push the drug further as he quickly showed signs of the toxic action of the drug. Louis Martin and Henri Darré†, in an article on the treatment of sleeping sickness at the Pasteur Hospital, believe that atoxyl can cure light forms of the disease, and even severe forms, but that it is often necessary to give large doses, viz., one gram, which imperil vision. These authors however, still admit that permanent cure is a doubtful matter.

In the experiments made by us we attempted, as far as possible, to administer a therapeutic dose of atoxyl. We first sub-inoculated four rats subcutaneously with *T. rhodesiense*. The lightest in weight, Rat 34, weighed 139 grams, and the heaviest weighed 256 grams, namely, Rat 37.

	Colour	Weight	Inoculation period	Duration of life	
Rat 34 ...	Piebald	139 grams	5 days	27 days	Animal House
Rat 35 ...	Piebald	145 grams	5 days	26 days	Cold Chamber
Rat 36 ...	White	251 grams	6 days	27 days	Cold Chamber
Rat 37 ...	White	256 grams	5 days	8 days	Animal House

All the four rats were adults. Two were piebald and two white, and all received, subcutaneously, the same dose of trypanosomes, namely, 200,000. Two of them, 35 and 36, were placed in the cold chamber, where the lowest temperature recorded was 20° F., and two were placed in the animal house. All these animals received atoxyl in solution subcutaneously in small repeated doses, which varied from $\frac{1}{80}$ to $\frac{1}{20}$ of a grain. Take the body weight of a man as being

* Proc. Roy. Soc., B. Vol. LXXXIII, 1911, pp. 206-211, and Ann. Trop. Med. and Parasit., Vol. IV, pp. 417-463.

† Bulletin of Sleeping Sickness, Vol. II, No. 18, 1910.

70 kilograms and the weight of our four rats as varying between 139 grams and 256 grams, and we find that the doses we administered to the rats, when calculated to the body weight of a man would represent doses varying between 4·55 grains and 25·14 grains. Rats 34 and 35, being the lighter rats, having received the same doses as Rats 36 and 37, therefore received a relatively larger dose when we consider the body weight. We now append tables of numbers of trypanosomes per c.mm. of peripheral blood and the doses of atoxyl given to Rats 34, 35, 36 and 37, and we also give graphs of the Rats 34, 35 and 36, which show the variations of the numbers of parasites in the peripheral blood.

RAT 34.—Piebald, weight 139 grams. Treated in Animal House with small doses of atoxyl.
Inoculation dose: 200,000 *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm. of blood		—	—	—	—	1,392	26,960
Atoxyl in grains	—	—	—	—	—	—	$\frac{1}{80}$

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm. of blood	40,460	8,118	1,440	10,500	5,888	101,200	96,800
Atoxyl in grains	$\frac{1}{80}$	—	$\frac{1}{60}$	—	—	$\frac{1}{60}$	—

Day	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm. of blood	73,920	4,000	20,496	5,796	12,696	3,864	19,136
Atoxyl in grains	$\frac{1}{30}$	—	$\frac{1}{30}$	—	$\frac{1}{20}$	—	$\frac{1}{30}$

Day	22	23	24	25	26	27	—
Number of Trypanosomes per c.mm. of blood	24,000	73,920	48,000	42,320	168,960	242,880	—
Atoxyl in grains	—	$\frac{1}{30}$	$\frac{1}{30}$	—	—	$\frac{1}{30}$	—

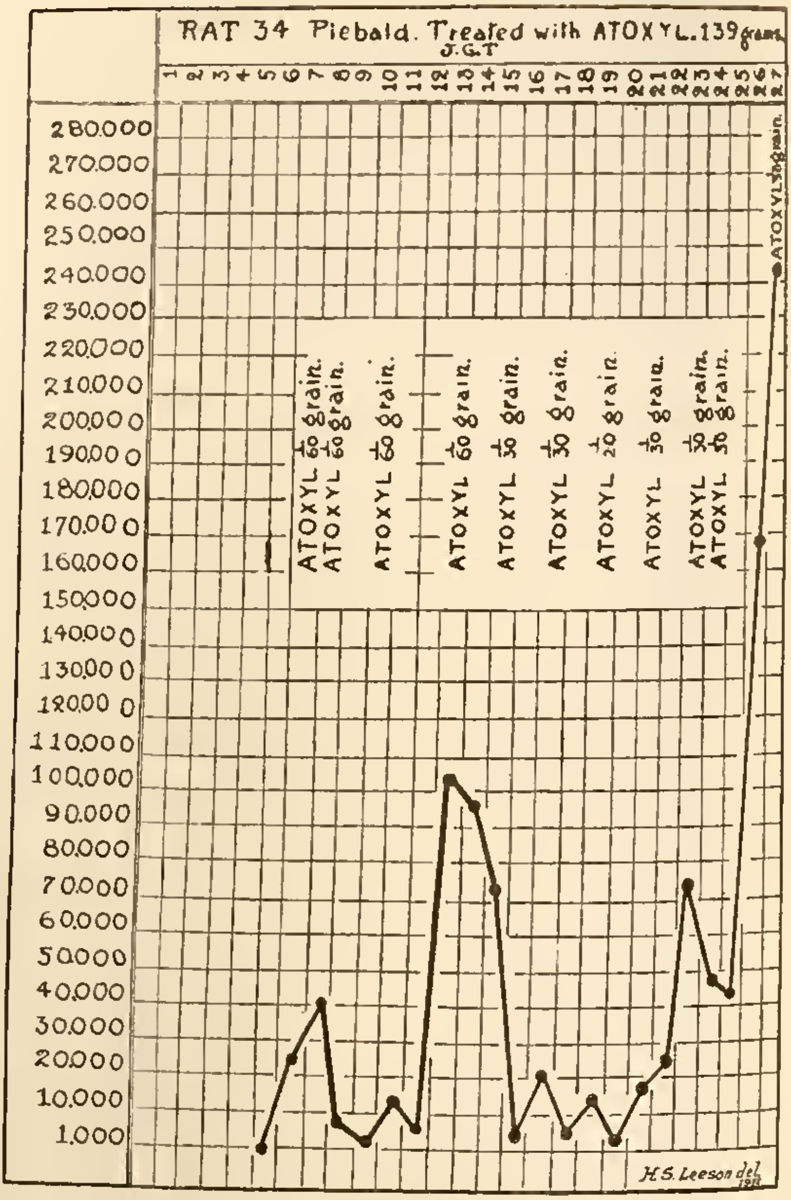


CHART 1.—Rat 34, inoculated with *T. rhodesiense*. Continuous line indicates the graph of the trypanosomes. Count of parasites made every 24 hours per c.mm. of peripheral blood.

Rat 35.—Piebald Adult Rat, weight 145 grams. Treated in Cold Chamber with small doses of atoxyl. Dose of inoculation: 200,000 *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm. of blood	—	—		—	—	2,064	25,112
Atoxyl in grains	—	—	—	—	—	—	1/60

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm. of blood	10,400	27,968	34,320	1,520	72,128	73,920	154,000
Atoxyl in grains	1/60	—	1/60	—	—	1/60	—

Day	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm. of blood	80,560	230,400	221.600	18,800	331,296	384,384	338,688
Atoxyl in grains	1/30	—	1/30	—	1/30	—	1/30

Day	22	23	24	25	26	—	—
Number of Trypanosomes per c.mm. of blood	390.000	550,000	447,680	383,040	570,000	—	—
Atoxyl in grains	—	1/30	1/30	—	—	—	—

Rat 36.—White Adult, weight 251 grams. Treated in Cold Chamber with small doses of atoxyl. Dose of inoculation: 200,000 *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm. of blood	—	—	—	—	—	—	192
Atoxyl in grains	—	—	—	—	—	—	—

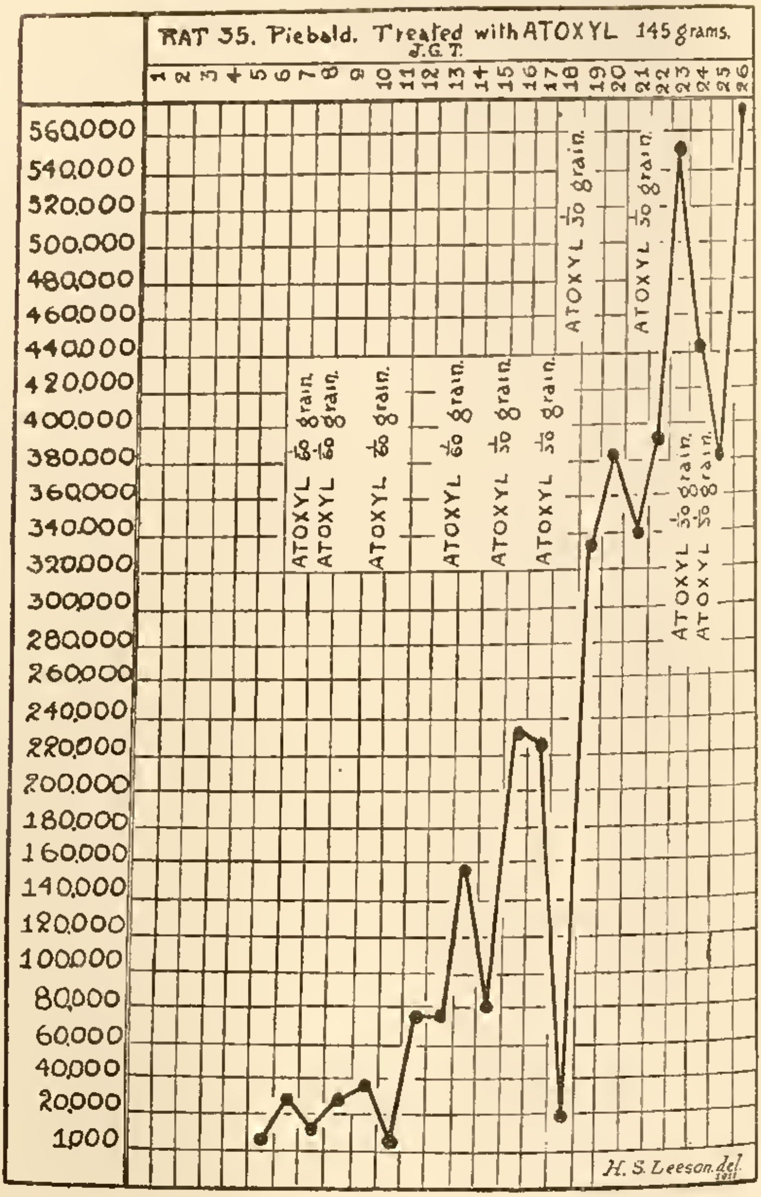


CHART 3.—Rat 35, inoculated with *T. rhodesiense*. Continuous line indicates the graph of the trypanosomes. Count of parasites made every 24 hours per c.mm. of peripheral blood.

Rat 36—continued.

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm. of blood	34,356	70,560	75,600	80,640	168,544	66,528	125,400
Atoxyl in grains	$\frac{1}{60}$	$\frac{1}{60}$	—	—	—	$\frac{1}{60}$	—

Day	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm. of blood	300,000	241,000	336,000	316,800	404,800	337,920	363,000
Atoxyl in grains	$\frac{1}{30}$	—	—	—	$\frac{1}{20}$	—	$\frac{1}{30}$

Day	22	23	24	25	26	27	—
Number of Trypanosomes per c.mm. of blood	410,000	281,600	138,000	530,000	483,840	500,000	—
Atoxyl in grains	—	$\frac{1}{30}$	$\frac{1}{30}$	—	—	$\frac{1}{30}$	—

Rat 37.—White, weight 256 grams, Adult. Treated in Animal House with small doses of atoxyl. Animal developed Pneumonia

Day	1	2	3	4	5	6	7	8
Number of Trypanosomes per c.mm. of blood	—	—	—	—	—	+	2,004	32,640
Atoxyl in grains	—	—	—	—	—	—	$\frac{1}{60}$	$\frac{1}{60}$

If now we examine table and chart of Rat 34 we find that the period between the crests of the waves representing the numbers of trypanosomes was certainly longer than in those of the untreated animals inoculated with *T. rhodesiense* (*vide* Paper on 'Enumerative Methods in Untreated Animals,' H. B. Fantham and J. G. Thomson*). We find there were two high crests in the graph of this animal corresponding to five days and ten days. The weight of this animal was 139 grams, and thus the doses administered were

* Proc. Roy. Soc., B. Vol. LXXXIII. 1911, pp. 206-211, and Ann. Trop. Med. and Parasit., Vol. IV. pp. 417-463.

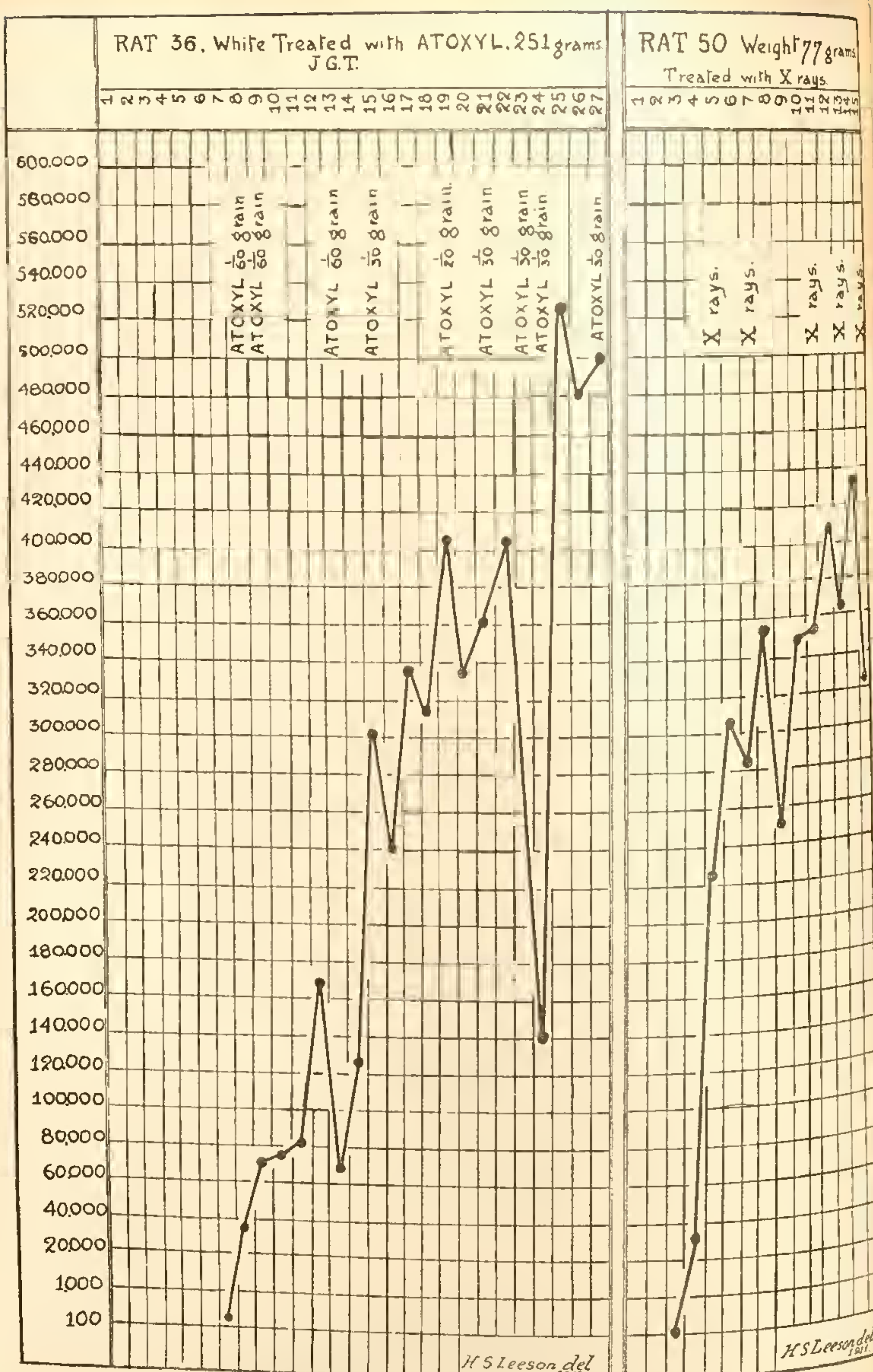


CHART 3.—Rats 36 and 50, inoculated with *T. rhodesiense*. Continuous line indicates the graph of the trypanosomes. Counts made every 24 hours per c.mm. of peripheral blood.

relatively large. The doses given may have had a slight trypanocidal action, but from the appearances of the smears we do not think there was any marked degeneration, and many trypanosomes were found actively dividing. The doses given to this animal would, if calculated to the body weight of a man of 70 kilograms, represent approximately 8·38 grains, 16·76 grains, and 25·14 grains. The lengthened period between the crests of the waves may have been caused by natural body resistance being raised. This animal lived twenty-seven days, whereas the longest period of life recorded in our controls, sub-inoculated with the same strain, was eighteen days in an animal weighing 48 grams.

If we examine now Rat 35, which was treated with similar doses of atoxyl, and kept in the cold chamber, we find quite a different character in the graph representing the numbers of trypanosomes in the peripheral blood. This animal was piebald in colour and weighed 145 grams, that is to say, it was only slightly heavier than Rat 34. This means that the doses given when calculated to the body weight of a man were smaller than those given to Rat 34. Here the doses would represent in man 8·04 grains, 16·08 grains and 24·12 grains. In spite of these doses the trypanosomes in the peripheral circulation rapidly increased in numbers with a staircase effect, and on the day before death reached the enormous number of 570,000 per c.mm. of blood. This animal lived twenty-six days, and received during that time ten doses of atoxyl. It cannot be said in this case that the atoxyl in the doses given had any trypanocidal action, but, again, the life of the animal was undoubtedly prolonged by the drug. The numbers of division forms seen in the smears of this animal showed that the proliferation of the trypanosomes was very active, and we also noted that the proportion of short, stumpy forms was also markedly increased.

Rat 36, white in colour, and treated in the cold chamber with small doses of atoxyl, shows a chart which in many respects simulates the chart of Rat 35. Here, again, we note the staircase rise of the trypanosomes in spite of continued treatment with small doses of atoxyl. This animal being heavier than Rats 34 and 35, received, therefore, doses which, when calculated to the body weight of a man, were relatively small, namely, 4·64 grains, 9·28 grains and 13·92 grains.

In this animal all the smears gave evidence of the trypanosomes being abnormally stimulated to division. Many of the trypanosomes were seen to be dividing into four, and the numbers before death reached 530,000 per c.mm. of blood.

This animal lived twenty-seven days. If we do not attribute the prolonged life of these three rats to the trypanocidal action of the atoxyl we must endeavour to explain it in another manner.

Arsenic in small doses affects metabolism and improves the general condition of the body. In small doses we find in man, both in health and disease, that the strength, weight and appetite are improved, and it is quite to be expected, therefore, that small repeated doses of atoxyl in rats would tend to raise the natural body resistance. In referring to Cushny's *Pharmacology* we find that Gies treated young rabbits with arsenic, and found that those treated weighed more, had larger bones, and more developed muscles than the untreated controls. It was also found that a pregnant female rabbit treated with arsenic gave birth to young rabbits of abnormal size. That arsenic, therefore, in small doses profoundly affects metabolism and improves the general condition we have good evidence, and this might possibly explain the lengthened life of the animals treated with atoxyl in small doses. In the same way the lengthened period between the crests of the waves in Rat 34 may possibly have been due to increased resistance of the body of the host rather than to a direct action of the drug administered upon the parasites.

In spite of the fact that the animals' lives were prolonged we had the trypanosomes increase steadily in numbers, and this was very marked in the cases of Rats 35 and 36. The numbers of trypanosomes in the peripheral blood remained consistently high, and the smears showed very active divisions of parasites. This can be attributed to the action of the atoxyl in small doses. We bave already referred to the general action of arsenic in small therapeutic doses. The drug seems to act as a tonic to the body in health as well as disease, and must, therefore, improve the condition of the cells of the whole body. Stockman and Greig* found the bone marrow in a state of unusual activity when arsenic was administered

* See Cushny's 'Pharmacology' on actions of arsenic.

in small doses, and there were greater numbers of red blood corpuscles.

Is it not possible, therefore, that atoxyl in small doses, such as given to these rats acted not only upon the cells of the body of the host, but on the trypanosomes themselves, and acted as a tonic to both, so stimulating increased division of the parasites? This would explain both the extraordinary division forms of the trypanosomes and also the prolonged life of the host without attributing to the atoxyl any trypanocidal action whatever.

It must be remembered, however, that we are working with a specially virulent strain of trypanosomes, and that, therefore, it may be peculiarly resistant to the action of atoxyl, which drug in many cases of human sleeping sickness seems undoubtedly to have a favourable influence on the disease.

Rats 35 and 36 were treated in the cold chamber and received atoxyl as well. In our experience, however, we find that although the cold tends to prolong the life of animals infected with trypanosomiasis to a slight extent it has not, as a rule, any marked effect upon the numbers of trypanosomes in the peripheral circulation, and thus we attribute the numerous division forms to the atoxyl.

(*b*) *Large doses of Atoxyl.*

We now tried the effect of a large dose of atoxyl on three rats, all heavily infected with *T. rhodesiense*.

Here we were guided in our experiments by the work of Thomas and Breinl*, who pointed out that if a cure was to be expected in animals a large dose of atoxyl was necessary.

RAT 38.—Piebald, weight 202 grams. (See Chart.) Dose of inoculation: 200,000 Trypanosomes. *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	—	202	6,162
Atoxyl 5 per cent. solution ...	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

* Memoir XVI, Trypanosomiasis research, pp. 52-57, Sch. of Trop. Med., Liverpool, October, 1905.

Rat 38—continued.

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	28,000	86,000	43,000	21,736	0	0	0
Atoxyl 5 per cent. solution ...	—	—	—	0·5 c.c.	—	—	—
Leucocytes	—	—	—	16,368	24.720	63,000	40,000

Day	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm.	0	1,600	18,000	32,000	120,000	98.000	89
Atoxyl 5 per cent. solution ...	—		—	—	—	0·5 c.c.	—
Leucocytes	22.880	—	—	—	—	12.360	24.000

Day	22	23	24	25	26	27	28
Number of Trypanosomes per c.mm.	320	4.510	12,000	26,000	60,000	23,600	20,310
Atoxyl 5 per cent. solution ...	—	—	—	—	—	—	—
Leucocytes	45,000	—	—	—	—	—	—

Day	29	30	31	32	33	34	35
Number of Trypanosomes per c.mm.	7.500	760	84	320	3.600	4,880	15.600
Atoxyl 5 per cent. solution ...	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	36	37	38	39	40	41	42
Number of Trypanosomes per c.mm.	23,000	79,488	123,600	193,000	177,744	350,000	144,000
Atoxyl 5 per cent. solution ...	—	—	—	—	—	—	—
Leucocytes	—	33,120	16,000	—	40,320	10,400	4.248

Rat 38—continued.

Day	43	44	45	46	47	48	49
Number of Trypanosomes per c.mm.	317,856	218,880	231,840	211,520	112,640	190,520	664,608
Atoxyl 5 per cent. solution ...	—	—	—	—	—	—	—
Leucocytes	21,924	—	31,020	9,280	26,048	19,404	33,120
Day	50	51	—	—	—	—	—
Number of Trypanosomes per c.mm.	816,960	1,500,000	—	—	—	—	—
Atoxyl 5 per cent. solution ...	—	—	—	—	—	—	—
Leucocytes	63,480	—	—	—	—	—	—

The above animal, Rat 38, lived fifty-one days. The control of this animal weighing 238 grams lived eighteen days, and the day before death had 350,000 trypanosomes per c.mm. Rat 38 received two doses of a 5 per cent. solution of atoxyl (0·5 c.c.) subcutaneously, and it will be noted that this dose calculated to the body weight of a man of 70 kilograms would be 130 grains approximately. It is of interest to note that the leucocytes rose in Rat 38 on the thirteenth day to 63,000 per c.mm.

Two other rats infected with the same strain received doses of 0·5 c.c. of a 5 per cent. solution of atoxyl.

Rat 40, white, weight = 129 grams.
Rat 41, white, weight = 113 grams.

Rat 40 had 4,800 trypanosomes in the cubic millimetre before injection, and next day we found 89 per c.mm., i.e., twenty-four hours after injection of atoxyl, and in forty-eight hours there were no trypanosomes in the peripheral circulation.

Rat 41 had 7,056 trypanosomes per c.mm. before injection of atoxyl and next day two, presumably dead trypanosomes, were found in a thick film. This rat, however, succumbed to the toxic action of the drug the following day.

Atoxyl, therefore, in large doses is a trypanocide. Arsenic in so-called poisonous doses causes adverse conditions in cells, and

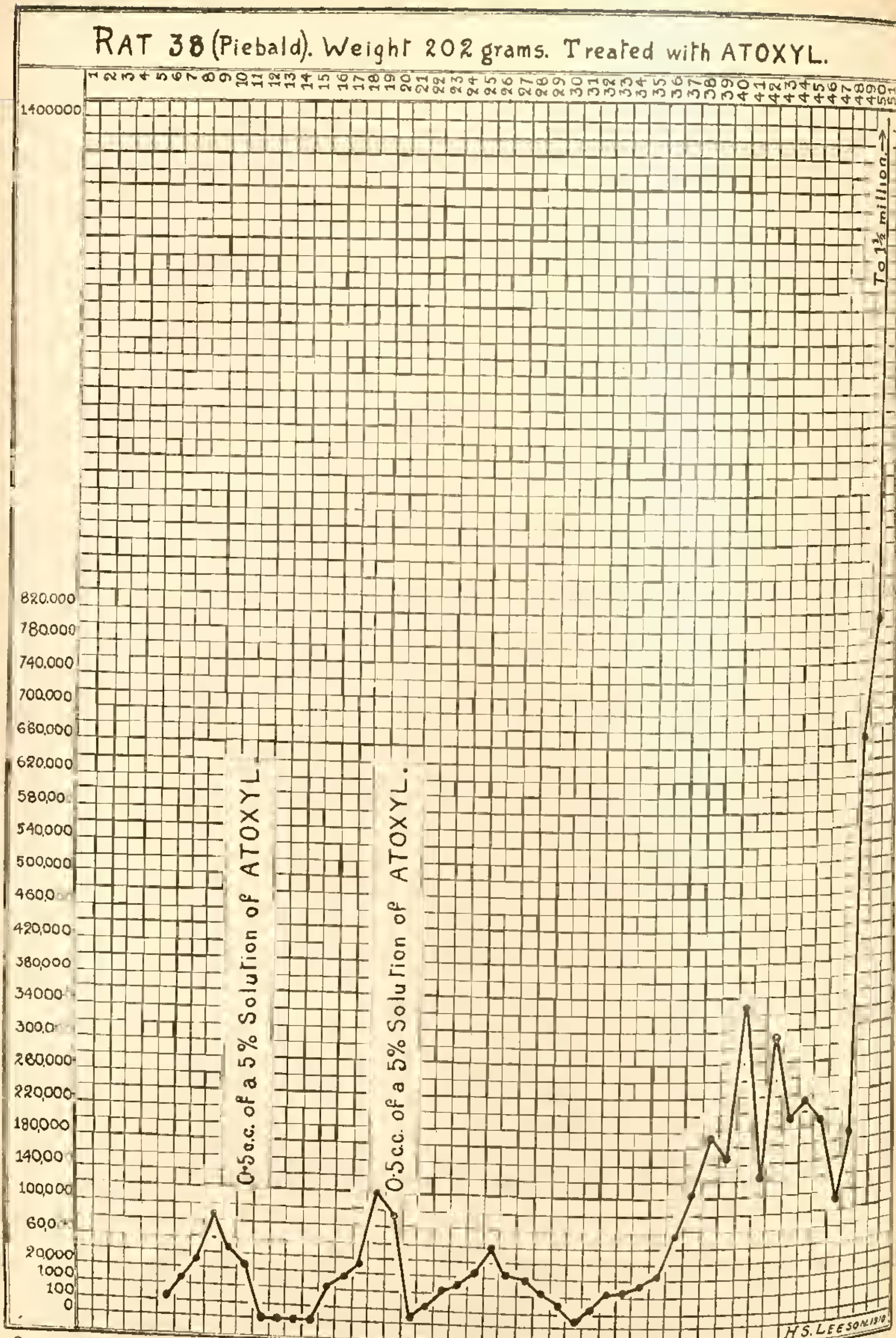


CHART 4.—Rat 38, inoculated with *T. rhodesiense.* Continuous line indicates the graph of the trypanosomes. Count of parasites made every 24 hours per c.mm. of peripheral blood.

we get fatty degeneration, especially of the cells of the liver and the epithelial cells of the stomach and intestines, and to a lesser extent in the kidneys and other organs in the body. If, therefore, we give atoxyl in a poisonous dose to an animal we kill not only the trypanosome cells, but also the body cells.

Unfortunately, in this strain of trypanosomes, even after the large dose of atoxyl given, the parasites appeared again in Rat 38 after an absence from the peripheral blood of four days. This is only to be explained by the resistant forms of Moore and Breinl (Ann. Trop. Med., 1, page 457 (1907)) forming in the spleen and bone marrow, and these again flagellate out under favourable circumstances.

Thomas and Breinl pointed out that to obtain a cure we must approach as near as possible to the lethal dose.

A small dose of atoxyl repeated did not act as a trypanocide, but we would suggest prolonged the life of the animal by acting as a tonic.

A large dose of atoxyl, on the other hand, is certainly a trypanocide, and clears the peripheral circulation of the parasites, but in this strain, *T. rhodesiense*, which is a particularly virulent one, the trypanosomes were evidently able to resist the action of the atoxyl by encysting themselves in the spleen and bone marrow (Moore and Breinl*).

OUR CONCLUSIONS ARE BRIEFLY THESE:—

(1) Small doses of atoxyl, i.e., therapeutic doses in this strain, viz., *T. rhodesiense*, prolong the life of the host, probably by raising the body resistance.

(2) Small doses of atoxyl in this strain of trypanosomes actually stimulate division of the parasites.

(3) Large doses of atoxyl kill the parasites, but probably do not cure the animal owing to atoxyl-resistant bodies (Moore and Breinl) being formed in the spleen and bone marrow.

(4) As has been noted by other observers in virulent forms of this disease, the initial dose must be large if a cure is to be expected.

(5) Atoxyl is *not* a specific in *T. rhodesiense*, but would appear to be almost as toxic to the body cells as to the trypanosomes themselves.

* Ann. Trop. Med. and Parasit., Vol. I. p. 457 (1907).

Treatment of T. rhodesiense in rats by subcutaneous injection of vaccines.

II. VACCINE TREATMENT

In view of the extensive development of vaccine treatment in disease, we have carried out some experiments in the treatment of trypanosomiasis. These experiments are few, and therefore inconclusive, but they have produced results of interest, and we are continuing the experiments in the hope of gaining further knowledge.

The vaccine used by us was not a true vaccine, because it contained serum, blood corpuscles, leucocytes, and the bodies of dead trypanosomes. It will thus be noted that we injected a rather complicated fluid, and that it was only a vaccine in the sense that it contained the bodies of dead trypanosomes.

The vaccine was prepared by searing the surface of the heart with a hot needle in order to get a sterile portion, and through this the blood was drawn into a sterile pipette and mixed with normal saline. This mixture was placed for half an hour in an incubator at 55° C., and finally a little trikresol was added. A count was made of the parasites in 1 c.mm. before they were subjected to heat. and appropriate dilution was carried out with normal sterile salt solutions.

There are two distinct points at which we may prepare this *so-called* vaccine. We may prepare it at the height of the rise of the trypanosomes in the peripheral blood, or it may be prepared when the trypanosomes are few in number.

In the former case we have a condition in which the numbers of trypanosomes are great, and probably the anti-bodies in the serum few. In the latter case we have, on the other hand, a condition where the trypanosomes are few and the serum rich in anti-bodies.

We have conducted experiments and used a so-called vaccine prepared at both the above stages. The first so-called vaccine was rich in trypanosomes, and was, therefore, more of the nature of a true vaccine than the latter, which was what we might call a vaccine *plus* an anti-serum.

We first experimented with a vaccine obtained at the height of the rise. Here it was noted that after subcutaneous injection a rise in the number of trypanosomes took place. The fact was first noted by D. Thomson, while conducting similar work on the human case of sleeping sickness. This observer asserts that an injection of a so-called vaccine—which was simply rats' blood, sterilised and mixed with normal saline—rich in the dead bodies of trypanosomes, stimulated the trypanosomes in the peripheral blood of the patient to divide; and the explanation seems to be based on the hypothesis of Dr. H. C. Ross, who holds that extracts of dead animal tissues stimulate cell division*.

In our experiments with rats infected with *T. rhodesiense*, we without exception found that when the blood of an animal rich in trypanosomes was injected subcutaneously, a rise of trypanosomes occurred during the next twenty-four hours. This rise was sometimes very sudden and exceedingly high, and if the dose was large and strong, the rise was (several times) so great as to cause the death of the animal. This is exactly in line with the treatment of disease with true bacterial vaccines, and here it is well known that if we treat say a mastitis with vaccine and give an excessive dose, we aggravate the condition. On the other hand, if a small dose was given to an infected rat, we got during the next twenty-four hours a rise, but not sufficient to kill the rat. This rise may well be represented as the negative phase, and a fall occurs usually in forty-eight to seventy-two hours, which may be considered as the positive phase.

We conclude, therefore, that the dose is important, i.e., it must not be too large, and secondly, we must at least allow an interval of one day before a second dose is administered.

In our first experiments we used six rats infected with *T. rhodesiense*, and vaccine was prepared from another rat, which was killed when the trypanosomes were at the height of a crest. We thus used a blood rich in trypanosomes, and relatively poor in anti-bodies. Three rats were kept as controls and three were treated. Two of those treated quickly succumbed, and we explain this by the fact that we gave two doses of vaccine without allowing an interval of at least twenty-four hours. We gave no time for a

* Brit. Med. Journ., June 11, 1910.

reaction to take place. The third rat, however, lived longer than the controls, and in this case, if we eliminate the fact that it may have been a more resistant animal, we had the life prolonged by the vaccine, which was given with an interval of one day between the doses.

We attempted the same line of treatment in two guinea-pigs, but here again, we cannot say that we prolonged life. We now attempted to obtain results with a blood prepared when the trypanosomes were low in the peripheral blood, i.e., we tried a condition in which one would expect to find anti-bodies, and in addition to this the blood was rich in leucocytes. The first injection, given on the eighth day, produced a fall in the trypanosomes and a rise in leucocytes which was very marked. The second injection was given on the thirteenth day, and again we obtained a fall (slight), but the leucocytes also fell. On the twentieth day we gave an injection of blood obtained from an animal when the trypanosomes were numerous; a very high rise took place and the animal died. This animal lived twenty-two days, i.e., we may conclude that we prolonged its life, and, indeed, the animal might have lived a longer period had we not on the twentieth day given too strong a dose of vaccine. We publish the chart of the last experiment (Rat 95), as it shows the rise and fall of the leucocytes in relation to the rise and fall of trypanosomes. It will be noted, if we refer to chart of Rat 95, that after inoculation a leucopenia takes place, and when trypanosomes first appear in the peripheral blood we have the leucocytes increasing. Again, it will be noted that when the trypanosomes are high in number that there tends to be a fall in the number of leucocytes, whereas when the trypanosomes are low in the peripheral blood there tends to be a marked leucocytosis. We have noticed this relation between leucocytes and trypanosomes in many cases.

We append tables of numbers of three untreated controls, viz., Rats 14, 15 and 16, and the tables of three rats treated with vaccine of a blood rich in trypanosomes, viz., Rats 42, 43 and 44, and, lastly, we append a chart of Rat 95 infected with *T. rhodesiense*. Here the first two doses of so-called vaccine were prepared from a rat when the trypanosomes were few in the peripheral blood, and we note that a fall in the numbers took place

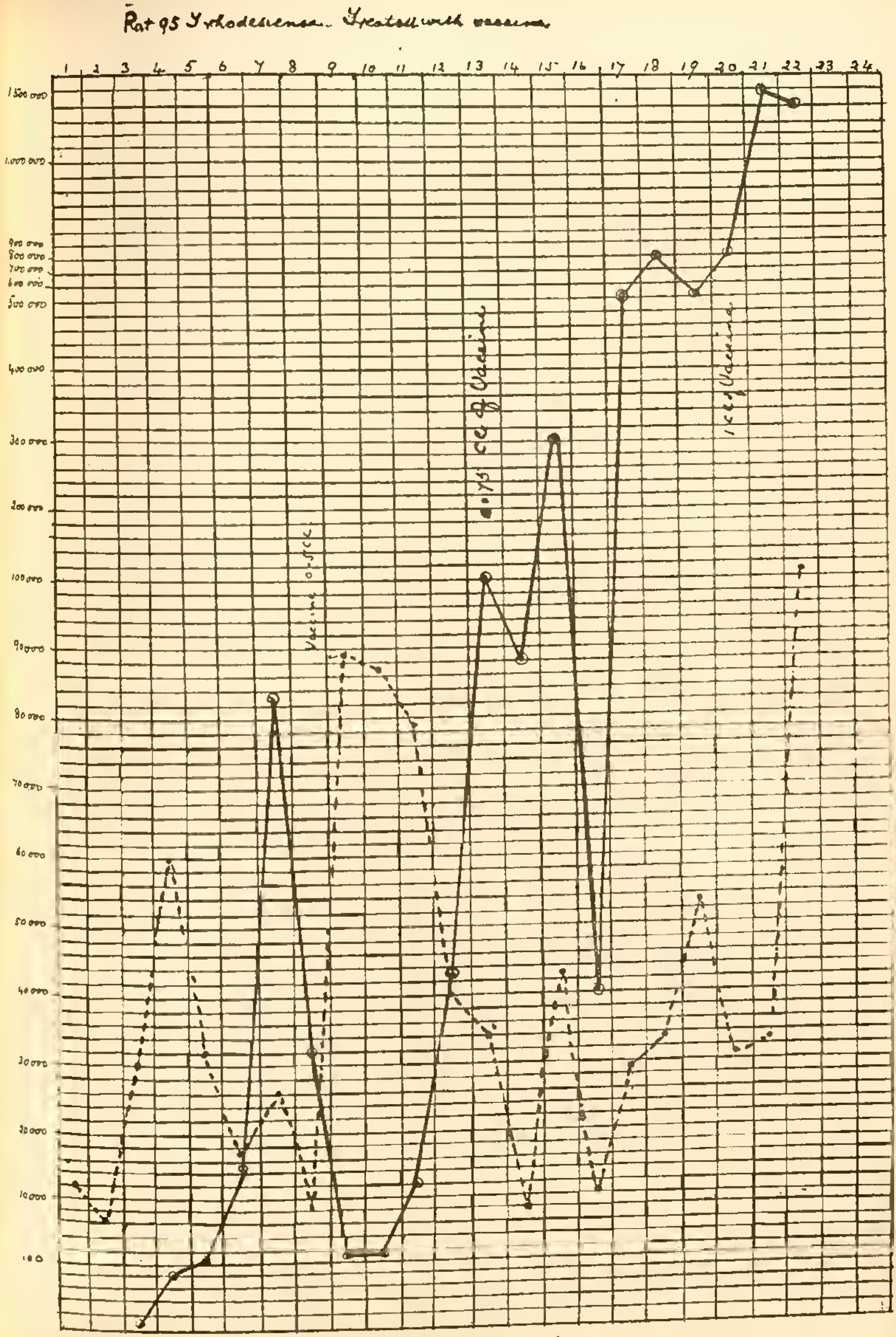


CHART 5.—Rat 95. ——— Continuous line indicates the trypanosome graph.
- - - - - - - Dotted line indicates the graph of leucocytes. Counts made per c.mm. of peripheral blood.

after these injections. The last injection of vaccine, given on the twentieth day, was prepared from a rat when the trypanosomes were very numerous, and we note that there was in the next twenty-four hours an enormous increase in the numbers of trypanosomes, from which the animals were evidently unable to recover.

CONTROLS—NO TREATMENT

RAT 14.—Piebald, weight 127 grams. Dose of inoculation: 200,000 Trypanosomes *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	1,440	45,600	66.240	7.144
Leucocytes	—	—	—	—	26,080	36,784	59,648

Day	8	9	10	11	12	—	—
Number of Trypanosomes per c.mm.	50,232	77,440	250,000	464,640	500,000	—	—
Leucocytes	—	—	—	—	—	—	—

RAT 15.—Piebald, weight 104 grams. Dose of inoculation: 200,000 Trypanosomes *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	4	4,620	45,440

Day	8	9	10	11	—	—	—
Number of Trypanosomes per c.mm.	24,472	44,352	194,128	410,000	—	—	—

RAT 16.—White, weight 58 grams. Dose of inoculation: 200.000 Trypanosomes *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—			104	37.000	30.240
Leucocytes	—	9,204	14.000	21,060	10.468	—	—
Temp.	—	42	44	36		44	46
Day	**8**	**9**	**10**	**11**	**12**	**13**	**—**
Number of Trypanosomes per c.mm.	126,000	86,640	121.600	180.000	160.800	No count	
Leucocytes					8,416	—	—
Temp.	34	52	20	34	—	18	—

RATS TREATED WITH VACCINE

RAT 42.—White, weight 121 grams. Dose of inoculation: 200,000 Trypanosomes *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—		—	—	—	90	24.000
Vaccine	—	—	—	—	—	—	—
Day	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Number of Trypanosomes per c.mm.	2.376	1,002	9.044	35,200	60,480	32,000	123,200
Vaccine	—	—	—	—	—	—	—

Day	15	16	17	18	19
Number of Trypanosomes per c.mm.	136,160	185.600	239,520	306,432	211,600
Vaccine	10,000,000	—	10,000,000	10,000,000	—

Rat 42—continued.

Day	20	21	—	—	—	—	—
Number of Trypanosomes per c.mm.	225,872	211,600	—	—	—	—	—
Vaccine			—	—	—		—

Rat 43.—White, weight 140 grams. Dose of inoculation: 200,000 Trypanosomes *T. rhodesiense*

Day	1	2	3	4	5	6
Number of Trypanosomes per c.mm.	—	—	—	840	52,000	214,016
Vaccine	—	—	—	—	12,000,000	12,000,000

Day	7	8	9	—	—	—
Number of Trypanosomes per c.mm.	144,000	310,000	5,292		—	—
Vaccine	—	—	—	—	—	—

Rat 44.—Piebald, weight 237 grams. Dose of inoculation: 200,000 Trypanosomes *T. rhodesiense*

Day	1	2	3	4	5	6
Number of Trypanosomes per c.mm.	—	—	65	35,296	42,320	248,960
Leucocytes	—	—	—	27,446	15,608	30,400
Vaccine	—	—	—	—	12,000,000	12,000,000

Day	7	8	9	10	11	—	—
Number of Trypanosomes per c.mm.	13,056	39,960	97,544	310,000	366,000	—	—
Leucocytes	43,000	—	—	—	—	—	—
Vaccine	—	—	—	—	—	—	—

III. TREATMENT IN THE COLD CHAMBER

The dimensions of the cold chamber are twelve feet long by seven feet wide by six and three-quarter feet high, and this is cooled by a refrigerator.

The lowest temperature reached in our experiments was 20° F. As the cooling apparatus was stopped during the night the temperature rose, and in the morning, before starting the machinery, reached 36° F. to 38° F., but this, of course, varied with the temperature of the atmosphere outside the chamber.

The humidity of the atmosphere in the chamber was low, and was found to vary between 50 and 60 per cent., but this also varied with the humidity outside, as the door had to be opened several times daily for the purposes of observation and to admit of feeding the animals.

For the above observations regarding humidity and temperature we are indebted to Major Williams, who compares the atmosphere of the cold chamber to that in the interior of Canada.

We were thus enabled to carry out treatment in a cold, dry atmosphere, and from personal experience we can testify to the invigorating feeling bestowed on us by a short sojourn in this chamber.

The animals in this chamber were active and took their food well, and, without doubt, seemed much better than their controls outside in the animal house.

The patient W.A., suffering from sleeping sickness contracted in Rhodesia, several times visited the cold chamber for treatment, but, unfortunately, at this time, no counts were made of the parasites in the peripheral circulation, but we have the evidence of the patient himself, who emphatically declared that he felt much better after being for some time in the cold chamber.

As the patient became worse, treatment had to be discontinued owing to the fact that the patient was considered too ill to travel from the Royal Southern Hospital to the University, and we had to resort to treatment in animals alone.

The animals used were guinea-pigs and rats, and we used two strains of trypanosomes—

(1) The *T. rhodesiense* obtained from patient W.A. (Ross and Thomson, 1910*), and

(2) An old laboratory strain of *T. gambiense*.

The essential differences between these two strains has been discussed by H. B. Fantham and J. G. Thomson in Paper on 'Enumerations in Untreated Animals suffering from Sleeping Sickness,' 1910†.

We at first selected guinea-pigs sub-inoculated with sleeping sickness, and from the tables we append it is evident that the animals were more resistant to the infection while living in the cold chamber.

T. gambiense. Old Laboratory Strain. Controls in Animal House

Animal	Incubation	Duration of life	Weight in grams
Guinea-pig 1[1]	4	28	495
Guinea-pig 2[1]	4	30	580
Guinea-pig 3[2]	4	105	534
Guinea-pig 4[2]	2	79	403

[1] Counts of these animals are not given.

[2] Daily counts of these animals are given in the paper on Enumerative Studies on Trypanosomes in Animals by Fantham and J. G. Thomson, where Guinea-pig 3 is numbered as 7 and Guinea-pig 4 is numbered as 6.

T. gambiense. Old Laboratory Strain. Treated in cold. Lowest Temp. 20° F.

Animal	Incubation	Duration of life	Weight in grams
Guinea-pig 5	19	102	648
Guinea-pig 6	6	93	562
Guinea-pig 7	16	74	623

* Proc. Roy. Soc., B, Vol. LXXXIII, pp. 187-205 (1911).

† Proc. Roy. Soc., B, Vol. LXXXIII, pp. 206-211 (1911), and Ann. Trop. Med. and Parasit., Vol. IV, pp. 417-463.

GUINEA-PIGS.—Sub-inoculated with *T. rhodesiense*

No.	Incubation	Duration of life	Weight in grams	
Guinea-pig 8 ...	13 days	120 days	350	Treated in Cold Chamber
Guinea-pig 9[1] ...	6 days	79 days	346	Treated in Animal House

[1] This is Guinea-pig 1 in paper on Enumerative Studies on Trypanosomes by Fantham and J. G. Thomson.

From the above tables it will be noted that the incubation period was delayed in all cases in the animals subjected to treatment in the cold chamber.

In the above tables Guinea-pigs 1, 2 and 3 were controls of Guinea-pigs 5 and 6.

Guinea-pig 4 was control of Guinea-pig 7, and Guinea-pig 9 was control of Guinea-pig 8.

The average incubation period of the five controls in the animal house was four days, whereas the average incubation period of the four animals treated in the cold chamber was $13\frac{1}{2}$ days.

The average duration of life in the controls was $64\frac{1}{5}$ days, whereas in the animals treated in the cold chamber the average life was $97\frac{1}{4}$ days.

In addition to this the animals in the cold were livelier, and took their food better than those in the animal house.

We can, therefore, conclude that in guinea-pigs sub-inoculated with both strains of sleeping sickness we had the incubation period delayed in the cold, and also the life of the animals prolonged when treated by cold.

We now experimented with rats. Here, again, we sub-inoculated the animals with the *T. rhodesiense* from the patient W.A., and we also sub-inoculated a series of rats with the old laboratory strain of *T. gambiense*.

CONTROLS. Old Laboratory Strain. *T. gambiense*

Number Piebald Rats	Weight in grams	Incubation	Duration of life	Number of Trypanosomes inoculated	Number of divisions in first 24 hours
Rat 23	173	6	19	2,000,000	8
Rat 27	113	5	15	60,000	7
Rat 28	101	5	17	60,000	3

Cold Chamber. Old Laboratory Strain. *T. gambiense*

Number Piebald Rats	Weight in grams	Incubation	Duration of life	Number of Trypanosomes inoculated	Number of divisions in first 24 hours
Rat 45	270	7	12	2,000,000	8
Rat 46	113	6	26	60,000	Fall in first 24 hours

Animal House. *T. rhodesiense*

Number White Rats	Weight in grams	Incubation	Duration of life	Number of Trypanosomes inoculated	Number of divisions in first 24 hours
Rat 17	62	4	8	38,000	A fall
Rat 16	58	4	13	200,000	8 divisions

Cold Chamber. *T. rhodesiense*

Number White Rat	Weight in grams	Incubation	Duration of life	Number of Trypanosomes inoculated	Number of divisions in first 24 hours
Rat 47	90	6	11	38,000	4 divisions

It will be noted from the above that the average incubation period of those in the cold chamber was 6·3 days, whereas the incubation period of the control was 4·8 days.

The average life in the cold was 16·3 days, and the average of those in the animal house was 14·2 days.

Rat 23 is the control of Rat 45. Rats 27 and 28 are controls of Rat 46. Rat 17 is the control of Rat 47.

We now append a table of the daily counts of the trypanosomes in the peripheral blood of animals treated in the cold. These counts were made every twenty-four hours. The incubation period is stated in days, and we define it as the period when trypanosomes are first found in one quarter cubic millimetre of peripheral blood.

The temperature in the tables is recorded according to the haematothermic scale of one of us (R.R.), where temp. = (F. − 95) × 10; F. being the temperature in degrees Fahrenheit recorded by a clinical thermometer. Temperature was taken in all cases per rectum. The counts of Guinea-pigs 1 and 2 are not given. For the counts of Guinea-pigs 3 and 4 see paper on 'Enumerative Studies on Trypanosomes,' by Fantham and J. G. Thomson, where Guinea-pig 3 is numbered as 7, and Guinea-pig 4 is numbered as 6.

GUINEA-PIG 5.—Cold Chamber. *T. gambiense.* Incubation period 19 days. Duration of life 102 days

Day	20	21	22	23	24	25	26	27
Number of Trypanosomes per c.mm.	4	16	53	28	12	24	32	40
Temp.	55	82	82	80	80	—	83	95
Weight in grams	—	—	648	—	—	—	—	—

Day	28	29	30	31	32	33	34
Number of Trypanosomes per c.mm.	92	1,060	2,208	5,568	7,108	10,476	4,806
Temp.	86	82	80	74	—	86	87
Weight in grams	—	603	—	—	—	—	—

Day	35	36	37	38	39	40	41
Number of Trypanosomes per c.mm.	4,192	160	408	200	—	300	684
Temp.	84	81	90	80	—	82	80
Weight in grams	—	608	—	—	—	—	—

Day	42	43	44	45	46	47	48
Number of Trypanosomes per c.mm.	—	—	1,040	1,520	—	—	2,880
Temp.	—	—	88	—	—	—	—
Weight in grams	—	—	—	—	—	—	—

Guinea-pig 5—continued.

Day	49	50	51	52	53	54	55
Number of Trypanosomes per c.mm.	5,344	—	—	21,252	—	19,136	9,700
Temp.	80	—	—	86	—	82	—
Weight in grams	—		—	—	—	—	—

GUINEA-PIG 6.—Cold Chamber. *T. gambiense*. Incubation period six days. Duration of life 93 days. Killed accidentally by CO_2 anaesthesia

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	—	—	Tryps. present
Temp.	—	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	—	—	—	—	—	—	—
Temp.	—	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm.	—	-	—	—	7,360	18,240	12,376
Temp.	—	—	—	—	—	65	82
Weight in grams	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Guinea-pig 6—continued.

Day	22	23	24	25	26	27	28
Number of Trypanosomes per c.mm.	17,304	18,384	29,348	9,200	4,032	3,488	2,640
Temp.	90	86	93	96	94	92	86
Weight in grams	562	—	—	—	—	—	—
Leucocytes	—	—	31,096	23,000	—	—	—

Day	29	30	31	32	33	34	35
Number of Trypanosomes per c.mm.	2,880	880	52	144	416	1,716	3,526
Temp.	94	88	—	91	97	88	86
Weight in grams	—	—	—	—	—	—	515
Leucocytes	—	—	—	—	—	—	—

Day	36	37	38	39	40	41	42
Number of Trypanosomes per c.mm.	4,366	10,504	—	15,420	13,380	—	—
Temp.	86	80	—	78	75	—	—
Weight in grams	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	43	44	45	46	47	48	49
Number of Trypanosomes per c.mm.	840	840	—	—	2,016	4,620	—
Temp.	80	—	—	—	—	78	—
Weight in grams	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	50	51	52	53	54	—	—
Number of Trypanosomes per c.mm.	—	24,128	—	66,700	57,120	—	—
Temp.	—	74	—	82	—	—	—
Weight in grams	—	—	—	—	—	—	—
Leucocytes	—	9,648	—	21,460	10,640	—	—

GUINEA-PIG 7.—Weight 623 grams. Cold Chamber. Dose of inoculation: 4,000,000. *T. gambiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	—	—	—
Leucocytes	—	—	48,000	—	—	—	—
Temp.	76	88	96	—	86	80	90
Weight in grams	623	—	—	—	—	—	594

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—
Temp.	84	90	82	—	82	80	80
Weight in grams	—	—	—	—	—	—	—

Day	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm.	—	—	40	0	156	176	426
Leucocytes	—	—	—	—	—	—	—
Temp.	80	80	79	—	85	80	78
Weight in grams	570	—	—	—	—	—	—

Day	22	23	24	25	26	27	28
Number of Trypanosomes per c.mm.	408	600	2,688	2,800	16,640	3,584	2,688
Leucocytes	—	—	—	—	—	—	—
Temp.	82	82	80	—	—	74	88
Weight in grams	—	—	—	—	—	—	—

Guinea-pig 7—*continued*.

Day	29	30	31	32	33	34	35
Number of Trypanosomes per c.mm.	600	2,448	11,852	13,104	15,708	14,900	9,680
Leucocytes	—	—	5,244	13,000	47,256	27,800	31,328
Temp.	84	—	86	—	80	74	84
Weight in grams	533	—	—	—	—	—	—

Day	36	37	38	39	40	41	42
Number of Trypanosomes per c.mm.	10,480	22,720	17.572	39,672	42,240	30,000	46,240
Leucocytes	24,160	15,920	33.396	15,200	—	39.672	—
Temp.	75	78	74	—	70	76	74
Weight in grams	—	—	—	—	—	—	—

Day	43	44	45	46	47	48	49
Number of Trypanosomes per c.mm.	195,000	108.000	36.000	46,880	39,700	55,524	68,040
Leucocytes	13,632	23,472	—	33,920	17,640	11,424	11,772
Temp.	82	53	86	—	70	70	82
Weight in grams	608	—	—	—	—	—	596

Day	50	51	52	53	54	55	56
Number of Trypanosomes per c.mm.	55,680	93,280	43,200	65,668	66,176	19,440	46,400
Leucocytes	4,000	8,500	—	16,588	—	—	—
Temp.	82	80	82	—	78	80	78
Weight in grams	—	—	—	—	—	—	—

Day	57	58	59	60	61	62	63
Number of Trypanosomes per c.mm.	52,376	70,056	28,800	44,800	21,160	37,296	62,160
Leucocytes	—	—	—	—	—	—	—
Temp.	50	80	81	—	—	95	85
Weight in grams	581	—	—	—	—	—	—

Guinea-pig 7—continued.

Day	64	65	66	67	68	69	70
Number of Trypanosomes per c.mm.	—	65,296	56,800	41,920	46,720	69,840	36,640
Leucocytes	—	—	—	—	—	—	—
Temp.	90	—	—	—	78	—	—
Weight in grams	622	—	—	—	—	—	—

Day	71	72	73	74	75	—	—
Number of Trypanosomes per c.mm.	70,664	64,584	21,672	6,880	Dead	—	—
Leucocytes	—	—	—	—	—	—	—
Temp.	—	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—

Guinea-pig 8.—Cold Chamber. Weight 350 grams. Dose of inoculation: 500,000 *T. rhodesiense*. Incubation period 13 days. Duration of life 120 days.

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	—	—	—
Leucocytes	—	35,000	—	18,304	29,456	32,208	10,208
Temp.	—	78	82	80	—	72	83
Weight in grams	—	350	—	—	—	—	—

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	—	—	—	—	—	—	8
Leucocytes	9,408	3,584	22,512	10,920	12,012	32,256	18,640
Temp.	83	68	80	76	—	74	82
Weight in grams	—	—	—	—	—	—	—

Guinea-pig 8—continued.

Day	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm.	2	8	4	12	16	40	8
Leucocytes	—	14,352	—	—	—	—	—
Temp.	80	82	76	77	—	70	76
Weight in grams	—	369	—	—	—	—	—

Day	22	23	24	25	26	27	28
Number of Trypanosomes per c.mm.	16	360	612	2,352	4,060	356	480
Leucocytes	—	11,760	21,692	—	30,740	—	—
Temp.	84	80	84	92	—	86	84
Weight in grams	—	387	—	—	—	—	—

Day	29	30	31	32	33	34	35
Number of Trypanosomes per c.mm.	6,440	6,720	3.192	4,320	6,216	13,376	9,760
Leucocytes	—	—	—	—	—	—	—
Temp.	86	80	81	79	—	—	92
Weight in grams	—	410	—	—	—	—	—

Day	36	37	38	39	40	41	42
Number of Trypanosomes per c.mm.	17,432	10,000	5,760	7,360	7,920	12,800	4,000
Leucocytes	—	—	—	—	—	—	—
Temp.	82	83	—	—	—	87	—
Weight in grams	—	443	—	—	—	—	—

Day	43	44	45	46	47	48	49
Number of Trypanosomes per c.mm.	7,632	2,784	40,152	9,240	8,064	10,184	14,240
Leucocytes	—	—	—	—	—	—	—
Temp.	—	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—

Guinea-pig 8—continued.

Day	50	51	52	53	54	55	56
Number of Trypanosomes per c.mm.	2,000	5,376	8,800	2,800	—	15,840	11,264
Leucocytes	—	—	—	—	—	—	—
Temp.	—	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—

CRYOTHERAPY EXPERIMENTS

RAT 23.—Piebald adult, weight 173 grams. Control in Animal House. Dose of inoculation: 2,000,000 *T. gambiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	—	—	120
Temp.	55	40	50	50	44	34	51
Leucocytes	—	—	29,000	—	—	—	—
Weight in grams	173	—	—	—	—	—	—

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	48,000	69,728	125,860	19,764	600	9,280	60,712
Temp.	45	65	50	—	50	66	38
Leucocytes	—	—	—	17,712	—	—	—
Weight in grams	167	—	—	—	—	—	—

Day	15	16	17	18	19	—	—
Number of Trypanosomes per c.mm.	130,000	142,000	100,000	250,000	164,000	—	—
Temp.	34	40	55	—	11	—	—
Leucocytes	—	—	—	—	—	—	—
Weight in grams	160	—	—	—	—	—	—

RAT 27.—White adult, weight 113 grams. Control in Animal House. Inoculation dose: 60,000 *T. gambiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	—	120	14,310
Temp.	61	49	32	12	44	—	5
Leucocytes	—	—	—	—	—	—	—
Weight in grams	113	—	112	—	—	—	—

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	101,740	146,880	69,400	23,276	55,224	67,096	57,552
Temp.	40	44	36	36	35	—	—
Leucocytes	—	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—

Day	15	—	—	—	—	—	—
Number of Trypanosomes per c.mm.	6,120	Dead	—	—	—	—	—
Temp.	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—

RAT 28.—White adult, weight 101 grams. Control in Animal House. Inoculation dose: 60,000 *T. gambiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	—	256	2,808
Temp.	59	70	43	42	40	—	56
Weight in grams	101	—	98	—	—	—	—

Rat 28—continued.

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	4,416	3,648	24,904	90,509	118,700	143,520	270,000
Temp.	44	42	34	26	24	—	40
Weight in grams	—	—	—	—	—	—	—

Day	15	16	17	18	—	—	—
Number of Trypanosomes per c.mm.	100,000	200,600	6,048	Dead	—	—	—
Temp.	78	42	—	—	—	—	—
Weight in grams	—	—	88	—	—	—	—

CRYOTHERAPY EXPERIMENTS

RAT 45.—Cold Chamber. Lowest temp. 20° F. Weight 270 grams. Dose of inoculation: 2,000,000 *T. gambiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	—	—	—
Temp.	54	70	60	—	56	52	60
Weight in grams	270	—	—	—	—	—	—
Leucocytes	—	—	45,000	—	—	—	—

Day	8	9	10	11	12	—	—
Number of Trypanosomes per c.mm.	92	24,012	480	430	1,100	—	—
Temp.	50	54	57	—	0	—	—
Weight in grams	246	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

RAT 46.—Cold Chamber. Weight 113 grams. Dose of inoculation: 60,000 *T. gambiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	—	—	3,608
Temp.	60	60	50	78	60	—	63
Weight in grams	113	—	111	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	3,456	2,352	8,640	2,400	7,100	3,104	13,024
Temp.	62	50	62	60	56	—	—
Weight in grams	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm.	40,120	63,480	11,448	61,560	16,416	28,188	46,980
Temp.	40	52	50	55	—	—	70
Weight in grams	—	—	125	—	—	—	—
Leucocytes	—	14,260	—	12,236	26,432	22,736	23,160

Day	22	23	24	25	26	27	—
Number of Trypanosomes per c.mm.	85,120	82,080	393,120	350,000	225,680	Dead	—
Temp.	68	63	34	58	50		
Weight in grams	—	—	—	—	—	—	—
Leucocytes	8,136	20,832	23,000	25,688	—		—

RAT 17.—White, weight 62 grams. Animal House. Dose of inoculation: 38,400 *T. rhodesiense*

Day	1	2	3	4	5	6	7	8
Number of Trypanosomes per c.mm.	—	—	—	—	1,500	500	1.380	4,028
Leucocytes	—	—	—	—	38,700	—	—	—
Temp.	—	50	30	56	50	—	—	70

RAT 16.—White, weight 58 grams. Animal House. Dose of inoculation: 200,000 *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	104	37,000	30,240
Leucocytes	—	9.204	14,000	21,060	10,468	30,800	28,000
Temp.	—	42	44	36	—	44	46

Day	8	9	10	11	12	13	—
Number of Trypanosomes per c.mm.	126,000	86,640	121,600	189,000	160,800	No count	
Leucocytes	18,584	27,360	16,340	38,124	8,416	—	—
Temp.	34	52	20	34	—	18	—

RAT 47.—Cold Chamber. Weight 90 grams. Dose of inoculation: 38,400 *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	—	—	5,184
Leucocytes	—	—	—	—	—	—	—
Temp.	—	50	45	50	40	—	—

Day	8	9	10	11	—	—	—
Number of Trypanosomes per c.mm.	82,818	230,000	106,000	330,000	—	—	—
Leucocytes	—	—	—	—	—	—	—
Temp.	40	66	—	36	—	—	—

The conclusions to be drawn from the above experiments are therefore in favour of treatment in the cold. The resistance of the animal is evidently raised, for the incubation period is delayed, and the animal lives longer in the cold. In favour of the cold also, we have the evidence of the patient, W.A., who said he felt better when in the cold chamber. What the physiological action of cold is we are not prepared in our present state of knowledge to state, but we certainly think that a person warmly clad is beneficially acted upon by a cold, dry atmosphere, and we would suggest that patients suffering from sleeping sickness would be greatly benefited by a sojourn, say, in Canada or Switzerland. The cold seems to be a valuable therapeutic agent in treatment.

We must acknowledge the fact that much more must be done in this investigation before definite conclusions are drawn, but the results have been so encouraging that we think they ought to be made known at once.

IV. X-RAYS

A young piebald rat, weighing 77 grams was inoculated with the Rhodesian strain of trypanosomes.

Here we have to thank Dr. Morgan, in charge of the Electrical Department at the Royal Southern Hospital, who kindly advised us in the administration of the rays. The exposures to the rays were given by Miss Wells, an experienced worker, under the supervision of Dr. Morgan. The rat lived fifteen days, and we can therefore say that this animal's life was prolonged, when compared with the average life of twenty-two untreated rats, which was 11·3 days.

In spite of five exposures, each of twenty minutes duration, during which period the whole body of the rat was exposed to the direct action of the rays, the trypanosomes remained lively and increased steadily in number in the peripheral circulation.

The rays were not, therefore, trypanocidal in the exposures given by us, but, curiously enough, the life of the animal was prolonged.

We append table :-

RAT 50.—Piebald, weight 77 grams. Dose of inoculation: 200,000 Trypanosomes *T. rhodesiense*

Day	1	2	3	4	5	6	7	8
Number of Trypanosomes per c.mm.	—	—	60	32,256	227,840	305,376	287,776	350,00
X-rays for 20 minutes	—	—	—	—	X-rays	—	X-rays	—

Day	9	10	11	12	13	14	15
Number of Trypanosomes per c.mm.	252,320	350,000	350,000	410,000	366,000	430,000	323,840
X-rays for 20 minutes	—	—	X-rays	—	X-rays	—	X-rays

The animal always seemed to brighten up during the exposure to the rays. The trypanosomes, it will be noted, increased in numbers in the peripheral circulation after each exposure, and we would conclude that there was no destruction of the trypanosomes. This is exactly in line with the experiments made by one of us (R.R.) several years ago, who found that exposure of the trypanosomes *in vitro* to the influence of X-ray had no trypanocidal action.

V. LEUCOCYTIC EXTRACT

This experiment was carried out at the suggestion of Dr. Moore Alexander, Pathologist to the Royal Southern Hospital, and he very kindly prepared the extract for us.

A white rat, weighing 120 grams, was inoculated with the Rhodesian strain of trypanosomes, and the disease was allowed to incubate. On the twelfth day, when the trypanosomes numbered 89,000 per c.mm., we injected subcutaneously 0·5 c.c. of leucocytic extract. The following day the trypanosomes numbered 220,800 per c.mm., and the leucocytes rose from 8,160 per c.mm. to 10,764 per c.mm. The animal lived fourteen days. We cannot, therefore, draw conclusions here, and much further work will have to be undertaken before we conclude as to the value of leucocytic extract in trypanosomiasis.

We append table:—

RAT 51.—White, weight 120 grams. Dose of inoculation: 200,000 Trypanosomes
T. rhodesiense

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	—		—	—	48	27,720	76,960	11.760	11,960	66.360	13.272	89,000	220,800	472.000
Leucocytes	—	—	—	5,680	—	—	19,040	—	40.296	—	—	8,160	10,764	—
Leucocytic extract	—	—	—	—	—	—	—	—	—	—	—	0.5 c.c.	—	—